Biology of Microorganisms

Sixth Edition

Thomas D. Brock
University of Wisconsin

Michael T. Madigan
Southern Illinois University

PRENTICE HALL
Englewood Cliffs, New Jersey 07632

Library of Congress Card Number: 90-14337

Book design and page layout: Thomas D. Brock
Cover design: Meryl Poweski
Editorial and production coordination:
Prentice Hall: Tom Aloisi
Science Tech: Carol Bracewell
Manufacturing buyer: Paula Massenaro

(Credits and acknowledgments appear on pp. 845 and 846, which constitute a continuation of the copyright page.)

Printed in the United States of America
10 9 8 7 6 5 4 3 2 1

ISBN 0-13-083817-9

Prentice-Hall International (UK) Limited, *London*
Prentice-Hall of Australia Pty. Limited, *Sydney*
Prentice-Hall Canada Inc., *Toronto*
Prentice-Hall Hispanoamericana, S.A., *Mexico*
Prentice-Hall of India Private Limited, *New Delhi*
Prentice-Hall of Japan, Inc., *Tokyo*
Simon and Schuster Asia Pte. Ltd., *Singapore*
Editora Prentice-Hall do Brasil, Ltda., *Rio de Janeiro*

Table 7.1 Kinds of mutants

Description	Nature f change	Detection of mutant
Nonmotile	Loss of flagella; nonfunctional flagella	Compact colonies instead of flat, spreading colonies
Noncapsulated	Loss or modification of surface capsule	Small, rough colonies instead of larger, smooth colonies
Rough colony	Loss or change in lipopolysaccharide outer layer	Granular, irregular colonies instead of smooth, glistening colonies
Nutritional	Loss of enzyme in biosynthetic pathway	Inability to grow on medium lacking the nutrient
Sugar fermentation	Loss of enzyme in degradative pathway	Do not produce color change on agar containing sugar and a pH indicator
Drug resistant	Impermeability to drug or drug target is altered or drug is detoxified	Growth on medium containing a growth-inhibitory concentration of the drug
Virus resistant	Loss of virus receptor	Growth in presence of large amounts of virus
Temperature sensitive	Alteration of any essential protein so that it is more heat sensitive	Inability to grow at a temperature normally supporting growth (e.g., 37°C) but still growing at a lower temperature (e.g., 25°C)
Pigmentless	Loss of enzyme in biosynthetic pathway leading to loss of one or more pigments	Detect visually by mutant being different color or colorless
Cold sensitive	Alteration in an essential protein so that it is inactivated at low temperature	Inability to grow at a low temperature (e.g., 20°C) that normally supports growth

7.2 The Molecular Basis of Mutation

As previously mentioned, mutations arise because of changes in the *base sequence* in the DNA. In most cases, mutations that occur in the base sequence of the DNA lead to changes in the organism; these changes are mostly harmful, although beneficial changes do occur occasionally.

Mutation can be either spontaneous or induced. **Spontaneous mutations** can occur as a result of the action of natural radiation (cosmic rays, etc.) which alter the structure of bases in the DNA. Spontaneous mutations can also occur during replication, as a result of errors in the pairing of bases, leading to changes in the replicated DNA. In fact, spontaneous mutations in any specific gene occur about once in every 10^6 to 10^{10} replications. Thus, in a normal, fully grown culture of organisms having approximately 10^8 cells/ml, there will probably be a number of different mutants in each milliliter of culture.

Point mutations

Point mutations are changes of *single* bases; an adenine base may be replaced by a guanine or thymine, for instance. What is the result of a point mutation? First, the error in the DNA is transcribed into mRNA, and this erroneous mRNA in turn is used as a template and translated into protein. The triplet code that directs the insertion of an amino acid via a transfer RNA will thus be incorrect. What are the consequences?

In interpreting the results of mutation, we must first recall that the genetic code is degenerate (see Section 5.9). Because of degeneracy, not all mutations in protein-encoding genes result in changes in protein. This is illustrated in Figure 7.3, which shows several possible results when a single *tyrosine codon* undergoes mutation. As seen, a point mutation from UAC to UAU would have no apparent effect, since UAU is an additional tyrosine codon. Such mutations are called **silent mutations**. Note that silent mutations almost always occur in the *third base* of the codon (arginine and

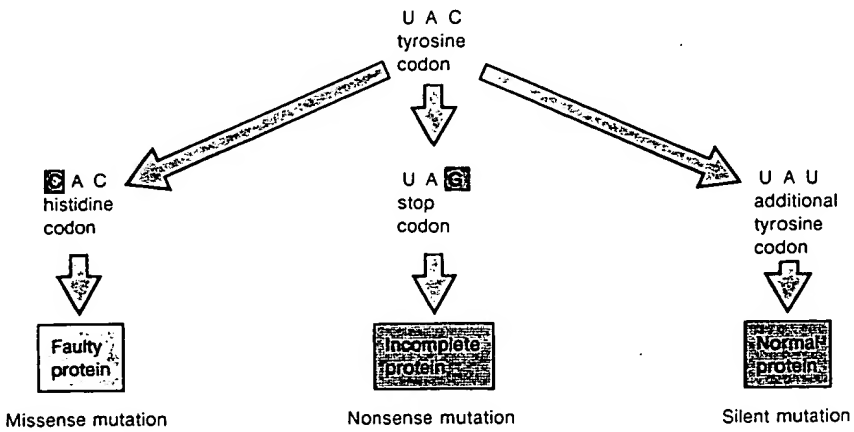

Figure 7.3 Possible effects of a point mutation in a gene coding for a protein: three different protein products from a single base change.

leucine can also have silent mutations in the first position). As seen in Table 5.5, changes in the first or second base of the triplet much more often lead to significant changes in the protein. For instance, a single base change from UAC to CAC (Figure 7.3) would result in a change in the protein from tyrosine to histidine. This is referred to as a **missense mutation**, because the chemical "sense" (sequence of amino acids) in the ensuing polypeptide has changed. If the change occurred at a critical point in the polypeptide chain, the protein could be inactive, or of reduced activity. Another possible result of a point mutation would be the formation of a *stop codon*, which would result in premature termination of translation, leading to an incomplete protein which would almost certainly not be functional (Figure 7.3). Mutations of this type are called **nonsense mutations** because the change is to a nonsense codon (see Section 5.9).

Thus, not all mutations that cause amino acid substitution necessarily lead to nonfunctional proteins. The outcome depends on where in the polypeptide chain the substitution has occurred, and on how it affects the folding and the catalytic activity of the protein.

Deletions and insertions

Deletions are due to elimination of portions of the DNA of a gene (Figure 7.4). A deletion may be as simple as the removal of a single base, or it may involve hundreds of bases. Deletion of a large segment of the DNA results in complete loss of the ability to produce the protein. Such deletions cannot be restored through further mutations, but only through genetic recombination. Indeed, one way in which large deletions are distinguished from point mutations is that the latter are usually reversible through further mutations, whereas the former usually are not.

Insertions occur when new bases are added to the DNA of the gene. Insertions can involve only a single base or many bases. Generally, insertions do not occur by simple copy errors as do deletions, but arise as a result of mistakes that occur during genetic recombination. Many insertion mutations are due to the insertion of specific identifiable DNA sequences 700 to 1400 base pairs in length called **insertion sequences** or **insertion elements**. The behavior of such insertion sequences is discussed in detail in Section 7.12.

Frame-shift mutations

Since the genetic code is read from one end in consecutive blocks of three bases, any deletion or insertion of a base results in a **reading-frame shift**, and the translation of the gene is completely upset (Figure 7.4). Partial restoration of gene function can often be accomplished by insertion of another base near the one deleted (one kind of suppressor mutation, see below). After correction, depending on the exact amino acids coded by the still faulty region and the region of the protein involved, the protein formed may have some biological activity or even be completely normal.

Polarity

As we have discussed, a cluster of genes in a procaryote may be transcribed together into a single mRNA. One consequence of the fact that translation occurs from one end is that nonsense mutations near the beginning of translation (the 5′ end) will terminate translation of all the successive genes, whereas nonsense mutations farther down will have fewer effects. This phenomenon is called **polarity**. Polarity gradients arise because when a ribosome reaches a chain-terminating nonsense codon, an incomplete polypeptide is released; the ribosome generally detaches from its mRNA template, so that the following genes are not translated. Because of polarity, nonsense mutations at the 3′ end will generally have no effect on genes transcribed at the 5′ end.

Back mutations or reversions

Many but not all mutations are reversible. A "revertant" is operationally defined as a strain in which the wild-type phenotype that was lost in the mutant is restored. Revertants can be of two types. In first-site (true) revertants, the mutation that restores activity occurs at the same site at which the original mutation occurred. In second-site revertants, the mutation occurs at some different site in the DNA.

Second-site mutations may revert because of several types of **suppressor mutations** that restore the original phenotype. Suppressor mutations are new mutations that suppress the effect of the original mutation. Several types of suppressor mutations are known: (1) a mutation somewhere else in the same gene can restore enzyme function, such as in a reading-frame shift mutation; (2) a mutation in another gene may

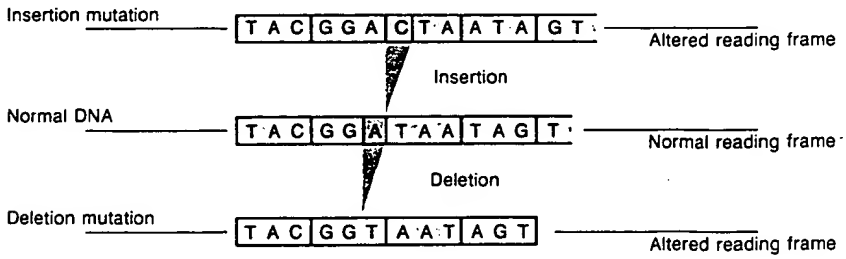

Figure 7.4 Shifts in reading frame caused by insertion or deletion mutations.

restore the wild-type phenotype; and (3) the mutation may result in the production of another enzyme that can replace the mutant one by introducing a metabolic pathway different from that used by the mutant enzyme. In this last type no production of the original enzyme occurs although it does in the other types.

Rates of mutation

There are wide variations in the rates at which various kinds of mutations occur. Some types of mutations occur so very rarely that they are almost impossible to detect whereas others occur so frequently that they often present difficulties for the experimenter who is trying to maintain a genetically stable stock culture.

Spontaneous mutations occur at frequencies around 10^{-6} per generation. This means that there is one chance in 1,000,000 that a mutation will arise at some location of a given gene during one cell cycle. Transposition events may occur more frequently, around 10^{-4}. On the other hand, the occurrence of a nonsense mutation is less frequent, 10^{-6}–10^{-8}, because only a few codons can mutate to nonsense codons. Missense mutations also occur at about the same frequency as nonsense mutations. Unless the mutant is selectable, the experimental detection of events of such rarity is obviously difficult and much of the skill of the microbial geneticist is applied to increasing the efficiency of mutation detection. As we will see in the next section, it is possible to significantly increase the rate of mutation by the use of mutagenic treatments.

Mutations, which can be either spontaneous or induced, arise because of changes in the base sequence of the DNA. A point mutation, which is due to a change in a single base, can lead to a single amino acid change in a protein, or to no change at all, depending on the particular codon involved. In a nonsense mutation, the codon becomes a stop codon and an incomplete protein is made. Deletions and insertions result in more dramatic changes in the DNA, including frame-shift mutations, and often result in complete loss of phenotype.

7.3 Mutagens

It is now well established that a wide variety of chemical and physical agents can induce mutations. We discuss some of the major categories and their actions here.

Chemical mutagens

An overview of some of the major chemical mutagens and their modes of action are given in Table 7.2. Several classes of chemical mutagens occur. A variety of chemical mutagens are **base analogs**, resembling DNA purine and pyrimidine bases in structure, yet showing faulty pairing properties (Figure 7.5). When one of these base analogs is incorporated into DNA, replication may occur normally most of the time, but occasional copying errors occur, resulting in the incorporation of the wrong base into the copied strand.

Table 7.2 Chemical and physical mutagens and their modes of action

Agent	Action	Result
Base analogs:		
5-Bromouracil	Incorporated like T; occasional faulty pairing with G	A–T pair → G–C pair Occasionally G–C → A–T
2-Aminopurine	Incorporated like A; faulty pairing with C	A–T → G–C Occasionally G–C → A–T
Chemicals reacting with DNA:		
Nitrous acid (HNO_2)	Deaminates A,C	A–T → G–C G–C → A–T
Hydroxylamine (NH_2OH)	Reacts with C	G–C → A–T
Alkylating agents:		
Monofunctional (e.g., ethyl methane sulfonate)	Put methyl on G; faulty pairing with T	G–C → A–T
Bifunctional (e.g., nitrogen mustards, mitomycin, nitrosoguanidine)	Cross-link DNA strands; faulty region excised by DNase	Both point mutations and deletions
Intercalative dyes (e.g., acridines, ethidium bromide)	Insert between two base pairs	Reading-frame shift
Radiation:		
Ultraviolet	Pyrimidine dimer formation	Repair may lead to error or deletion
Ionizing radiation (e.g., X rays)	Free-radical attack on DNA, breaking chain	Repair may lead to error or deletion